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LAPAROTOMY ENHANCES THE PRODUCTION OF REACTIVE NITROGEN SPECIES IN THE HEARTS OF RATS EXPOSED TO A SINGLE PROLONGED STRESS

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This study is a part of the research project "The role of transcription factors, circadian oscillator system and metabolic disorders in the formation and functioning of pathological systems" (State registration number 0119U103898).

The aim of this study was to assess the production of reactive nitrogen species in the hearts of rats subjected to surgical trauma simulated against the background of post-traumatic stress disorder (PTSD).

Materials and methods. The study was conducted on 42 white Wistar rats weighing 210-230 g, then divided into 6 groups: Group 1 consisted of intact animals, Group 2 included animals exposed to the induction of the PTSD model through single-prolonged stress (SPS), Group 3 – rats subjected to a sham operation, Group 4 – animals undergoing laparotomy, Group 5 – rats undergoing a sham surgical operation following SPS, and Group 6 – animals undergoing laparotomy under modeled SPS. NO synthase activity in the heart homogenate was determined spectrophotometrically.

Results. The formation of peroxynitrite was assessed by the content of peroxynitrite of alkaline and alkaline-earth metals. SPS exposure significantly increases the production of reactive nitrogen species in the hearts of rats, in particular, it enhances NO synthase activity by activating the inducible isoform and reduces the activity of constitutive NO synthases, which is accompanied by the growth in the concentration of peroxynitriles. On the 7th day after laparotomy against the background of experimental PTSD model, the indicators of nitrosative stress in the hearts of rats (total and inducible NO synthase activity and peroxynitrite concentration) significantly exceeded their values in the groups subjected to a single laparotomy and to a sham operation against the background of simulated SPS.

Key words: single prolonged stress, posttraumatic stress disorder, surgical trauma, laparotomy, reactive nitrogen species, NO synthase, peroxynitrite, nitrosative stress, heart, rats.

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Introduction
The traditional focus on psychological symptoms in diagnosing post-traumatic stress disorder (PTSD) may be insufficient. An expanding body of research suggests a pivotal connection between this disorder and disruptions in the immune system. Notably, epidemiological studies demonstrate a significant correlation between PTSD and a group of physical illnesses characterized by a systemic inflammatory response (SIR), including conditions like metabolic syndrome and cardiovascular diseases [1]. Supporting the connection between PTSD and SIR, several recent studies on blood biomarkers have identified significantly elevated levels of pro-inflammatory markers, including interleukin-1β, interleukin-6, and tumour necrosis factor-α, and C-reactive protein, in individuals with PTSD compared to healthy controls. Furthermore, numerous animal and human studies suggest that SIR not only coexists with PTSD but also plays a crucial role in its development and progression [1, 2]. Emerging studies elucidate the complex interaction between imbalances in oxidative and nitrosative stress and heart dysfunction within the context of systemic inflammatory response (SIR) [3]. This intricate puzzle, presumably underlying the resistance to the therapy for heart failure, prompts an investigation into antioxidant therapies as a potential strategy for reducing mortality. SIR is also known as a contributive factor to the pathogenesis of surgical trauma [4]. In certain susceptible individuals, this can lead to the development of multiple organ failure syndrome and, ultimately, death. Even a single laparotomy can induce a pro-inflammatory phenotype characterized by neuroendocrine stress, cortical excitability, immune activation, metabolic changes, and coagulopathy [5].

Nevertheless, the specific role of the SIR and nitrosative stress in the mechanistic pathways of the pathogenic effects of surgical trauma under PTSD remains unclear. Addressing these issues is important for elaborating new medical technologies focused on ensuring the safety of surgical interventions.

The aim of this study is to assess the production of reactive nitrogen species in the hearts of rats subjected to surgical trauma simulated against the background of PTSD.

Material and methods
The study involved 42 white Wistar rats weighing 210-230 g, then divided into 6 groups: Group 1 consisted of intact animals, Group 2 included animals exposed to the induction of the PTSD model through single-prolonged stress (SPS), Group 3 – rats subjected to a "sham" operation, Group 4 – animals undergoing laparotomy, Group 5 – rats undergoing a sham surgical operation following SPS, and Group 6 – animals undergoing laparotomy under SPS.

The study was conducted in accordance with the requirements of the European Convention for the protection of vertebrate animals used for research and other scientific purposes (Strasbourg, 1986) and approved by the Commission on Bioethics of Poltava State Medical University (Minutes No. 213, 22.02.2023). The rats were euthanized seven days after undergoing either sham surgery or laparotomy under thiopental anaesthesia. Sodium thiopental (“Arterium”, Ukraine) was administered intraperitoneally in a dose of 50 mg/kg.

The SPS procedure included sequential exposure to multimodal stressors [6]. The rats were immobilized for 2 hours on a metal plate, fixing their limbs with surgical tape and restricting head movements. After that, the animals were subjected to forced swimming in a plexiglass cylinder filled 2/3 with fresh water at 24°C. After swimming, the rats were dried with a hair dryer and allowed 15 min to recover before being exposed to ether vapour in a glass jar until they lost consciousness. After that, all animals were placed two by two in cages and left alone for 7 days.

The procedure of the sham surgery included anaesthesia, hair removal, fixation of the animals, and compression of the abdominal skin with a Mikulich clamp with a single click without creating a surgical wound.

Surgical trauma was induced through a laparotomy procedure conducted under intraperitoneal ketamine anaesthesia in a dosage of 7 mg/kg per 1 kg of body weight. Surgical intervention commenced following the onset of narcotic sleep and the attainment of a necessary level of anaesthesia. After shaving the surgical site and applying an antiseptic solution, a 1 cm long linear incision was made in the hypogastrium. Subsequently, the muscles, fascia, and peritoneum were dissected, and a small intestine loop was extruded through this incision. The intestine was stimulated by massaging movements of the index finger and thumb for 10 seconds [6]. The intestine was then returned to the abdominal cavity, and the incision was sutured in layers using polyglycolide sutures, followed by antiseptic treatment.

The activity of total NO synthase (NOS) [7] and its constitutive isoform (cNOS) [8] in the heart homogenate was determined spectrophotometrically. The activity of the inducible isoform of this enzyme (iNOS) was assessed by the formula: iNOS activity = total NOS activity – cNOS activity. The formation of peroxynitrite was measured by the content of peroxynitrite of alkaline and alkaline-earth metals [9]. The data obtained were processed using the Student's test.
Results

SPS exposure significantly changed the activity and ratio of different NOS isoforms in rat heart homogenate (Table). Under these conditions, total and inducible NO synthase activity increased by 55.0% and 73.7%, respectively (all at P<0.001). The activity of cNOS decreased by 61.2% (P<0.01). The concentration of peroxynitrite increased by 29.0% (P<0.001).

<table>
<thead>
<tr>
<th>Groups</th>
<th>NO synthase activity, μmol NO/min·g protein</th>
<th>Peroxynitrite content, μmol/g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Constitutive</td>
</tr>
<tr>
<td>Intact animals</td>
<td>3.53±0.21</td>
<td>0.49±0.05</td>
</tr>
<tr>
<td>SPS</td>
<td>5.47±0.35 *</td>
<td>0.19±0.03 *</td>
</tr>
<tr>
<td>Sham operation</td>
<td>3.83±0.71 **</td>
<td>0.43±0.10 **</td>
</tr>
<tr>
<td>Laparotomy</td>
<td>5.03±0.51 *</td>
<td>0.25±0.08 *</td>
</tr>
<tr>
<td>Sham operation + SPS</td>
<td>4.18±0.57</td>
<td>0.37±0.09</td>
</tr>
<tr>
<td>Laparotomy + SPS</td>
<td>7.42±0.28</td>
<td>0.22±0.07 *</td>
</tr>
</tbody>
</table>

Note: * P<0.05 compared to the results of the 1st group, ** 2nd group, *** 3rd group, **** 4th group, ***** 5th group.

Table
Indicators of reactive nitrogen species production in rat heart homogenate under conditions of surgical trauma reproduced against the background of post-traumatic stress disorder (M±m)

In sham-operated animals, the activity and ratio of NOS isoforms did not exhibit significant changes compared to the control, except for a 16.7% increase in the concentration of peroxynitrite (P<0.05).

The laparotomy was accompanied by an increase in total and induced NOS activity by 42.5% (P<0.02) and 57.2% (P<0.01), respectively, with a decrease in cNOS activity by 49.0% (P<0.05). The content of peroxynitrites increased by 24.6% (P<0.01).

On day 7 after the sham operation in rats previously exposed to SPS, NO synthase activity (both total and its isoforms) did not differ significantly from the findings in the comparison groups. However, the concentration of peroxynitrite in the heart homogenate increased by 34.8% (P<0.001), but did not differ significantly from the value of group 2. At the same time, compared with the values of the 3rd group, an increase in this indicator was observed by 15.5% (P<0.05).

On the 7th day following laparotomy under SPS exposure, total and inducible NO synthase activity increased by 2.1 and 2.4 times, respectively (both at P<0.001), compared to the results in group 1. These values were 35.6% and 36.4% higher (both at P<0.001) than the findings in group 2, and 47.5% (P<0.01) and 50.6% (P<0.001) higher compared with the results in group 4. Furthermore, NOS and iNOS activity under these conditions exceeded the corresponding values of group 5 by 77.5% and 88.5% (both at P<0.001). The activity of cNOS in animals of group 6 decreased by 55.1% (P<0.01) compared with the control, but did not differ significantly from the values of other comparison groups.

The content of peroxynitrites increased by 71.0% (P<0.001) compared to the control. This result exceeded the findings of group 2 by 32.6% (P<0.001) and group 4 by 37.2% (P<0.001). At the same time, the concentration of peroxynitrite was 26.9% (P<0.001) higher than the corresponding result of group 5.

Discussion

SPS, serving as a model of PTSD, is associated with an elevation in both total and inducible NO synthase activity, as well as an increase in the concentration of the highly toxic active form of nitric oxide, peroxynitrite, in rat heart homogenate. These findings provide justification for confirming the occurrence of nitrosative stress in heart tissue – a crucial indicator of a systemic inflammatory response accompanied by the activation of redox-sensitive transcription factors [10, 11]. Recent research has demonstrated a correlation between the severity of PTSD and depressive symptoms with the development of oxidative-nitrosative stress [12]. This pathology has also been linked to pro-inflammatory NF-κB signalling and glucocorticoid resistance in monocytes [13].

SPS, serving as a model of PTSD, is accompanied by an increase in total and inducible NO synthase activity as well as in the concentration of the highly toxic active form of nitric oxide, peroxynitrite, in rat heart homogenate. These findings provide grounds for confirming the development of nitrosative stress in heart tissue, which is a crucial indicator of a systemic inflammatory response accompanied by the activation of redox-sensitive transcription factors [10, 11]. Recently research has demonstrated that the severity of PTSD and depressive symptoms correlates with the development of oxidative-nitrosative stress [12]. This pathology has also been linked to...
pro-inflammatory NF-κB signalling and glucocorticoid resistance in monocytes [13].

In the case of prolonged and/or intense PTSD, antioxidant mechanisms are insufficient to provide neuroprotection, which ultimately leads to oxidative-nitrosative stress. At the same time, numerous triggers of inflammation are activated and/or influenced by redox systems in a closely interconnected manner [14]. According to our data, the severity of oxidative-nitrosative stress in heart tissues increased after surgical trauma (laparotomy) in rats previously exposed to SPS. Previously, similar changes were recorded in the reproduction of a lipopolysaccharide-induced model of systemic inflammatory response, which the authors attributed to an imbalance between the activation of the transcription factors NF-κB and Nrf2 [15].

Prospects for further research include elucidating the role of redox-sensitive transcription factors in the mechanisms of surgical trauma complications formation under conditions of systemic inflammatory response associated with PTSD.

Conclusions
1. Replication of an experimental model of post-traumatic stress disorder (single prolonged stress) significantly increases the production of reactive nitrogen species in the rat heart. This includes an elevation in NO synthase activity through the activation of the inducible isofrom and a reduction in the activity of constitutive NO synthases, coupled with an increase in the concentration of peroxynitrates of alkaline and alkaline-earth metals.

2. On the 7th day following laparotomy, after the replication of the experimental model of post-traumatic stress disorder, the indices of nitrosative stress in the rat heart (total and inducible NO synthase activity and peroxynitrite concentration) markedly surpass their levels observed after a single laparotomy and a sham operation against the background of simulated single prolonged stress.

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Conflict of interest:
The authors declare no conflict of interest

Ethical approval:
Ethics approval and consent to participate the study was approved by the Committee on Bioethics and Ethical Issues of Poltava State Medical University (Minutes No. 213, 22.02.2023).

References
ЛАПАРОТОМІЯ ПОСИЛЮЄ ПРОДУКЦІЮ АКТИВНИХ ФОРМ АЗОТУ В СЕРЦІ ЩУРІВ ПІСЛЯ ВІДТВОРЕННЯ ОДНОКРАТНОГО ТРИВАЛОГО СТРЕСУ

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Метою дослідження було визначення продукції активних форм азоту в серці за умов хірургічної травми, відтвореної на тлі посттравматичного стресового розладу (PTSD, post-traumatic stress disorder).


Результати. Відтворення SPS суттєво збільшує продукцію активних форм азоту в серці щурів: підвищує активність NO-синтази за рахунок активації індуцибельної ізоформи, знижує активність конститутивних NO-синтаз, що супроводжується зростанням концентрації пероксинітритів. На 7-му добу після лапаротомії на тлі відтворення експериментальної моделі PTSD показники нітрозативного стресу в серці щурів (загальна та індуцибельна NO-синтазна активність і концентрація пероксинітритів) достовірно перевищують їх значення після окремої лапаротомії і після «хибної» операції на тлі модельованого SPS.

Ключові слова: одноразовий тривалий стрес, посттравматичний стресовий розлад, хірургічна травма, лапаротомія, активні форми азоту, NO-синтаза, пероксинітрит, нітрозативний стрес, серце, щури.